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Non-nucleotide linking reagents for nucleotide probes.

Abstract:

A versatile reagent with a non-nucleotide monomeric unit having a ligand, and first and second coupling groups which are linked to the non-nucleotide monomeric unit. The ligand can be either a functional moiety, such as a label or intercalator, or a linking arm which can be linked to such a moiety. Such reagent permits preparation of versatile nucleotide/non-nucleotide polymers, having any desired sequence of nucleotide and non-nucleotide monomeric unit

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s, each of the latter of which bear a desired ligand. These polymers can for example, be used as probes which can exhibit enhanced sensitivity and/or which are capable of detecting a genus of nucleotides each species of which has a common target nucleotide sequence of interest bridged by different sequences not of interest.

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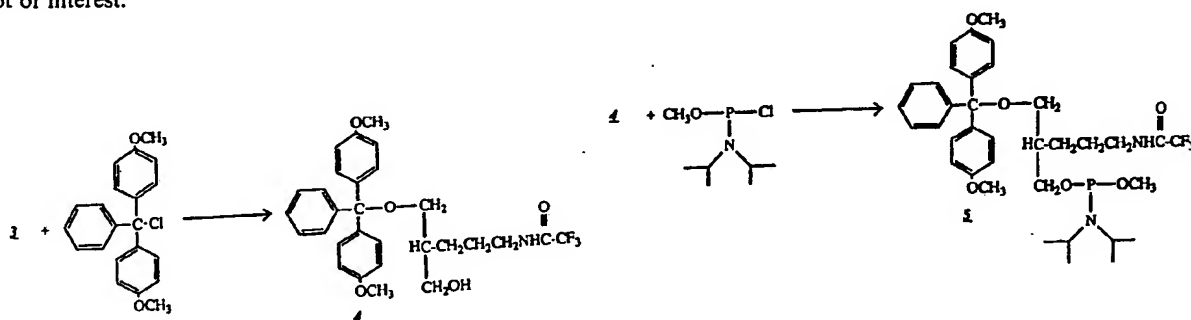
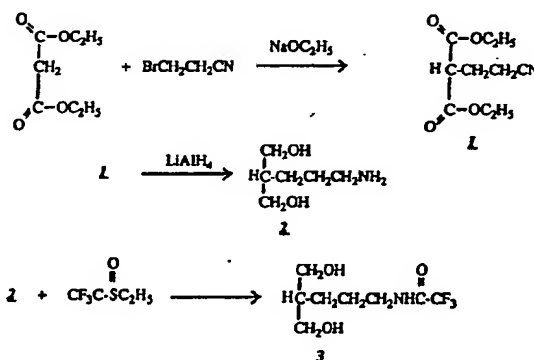


## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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**(54) Title:** NON-NUCLEOTIDE LINKING REAGENTS FOR NUCLEOTIDE PROBES**(57) Abstract**

A versatile reagent with a non-nucleotide monomeric unit having a ligand, and first and second coupling groups which are linked to the non-nucleotide monomeric unit. The ligand can be either a functional moiety, such as a label or intercalator, or a linking arm which can be linked to such a moiety. Such reagent permits preparation of versatile nucleotide/non-nucleotide polymers, having any desired sequence of nucleotide and non-nucleotide monomeric units, each of the latter of which bear a desired ligand. These polymers can for example, be used as probes which can exhibit enhanced sensitivity and/or which are capable of detecting a genus of nucleotides each species of which has a common target nucleotide sequence of interest bridged by different sequences not of interest.



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## AMENDED CLAIMS

[received by the International Bureau on 7 April 1989 (07.04.89);  
original claims 1,3,10,11,15,16,18,19,23,25,31,33-35 and 43 amended; new claims  
49 and 50 added; other claims unchanged (16 pages)]

1. (amended) A non-nucleotide reagent suitable for  
preparing a nucleotide/non-nucleotide polymer, comprising:

- 5 (a) a non-nucleotide monomeric unit, having a  
ligand which is selected from a side arm  
with an attached chemical moiety, and from  
a linking arm which can be activated under  
non-adverse conditions so as to be capable  
of linking with a chemical moiety;
- 10 (b) first and second non-nucleotide coupling  
groups linked to the monomeric unit, the  
first coupling group of which is capable of  
coupling the non-nucleotide monomeric unit  
to a first additional monomeric unit, while
- 15 the second coupling group remains inac-  
tivated so as to be substantially incapable  
of coupling, but can thereafter be ac-  
tivated under non-adverse conditions to  
couple the non-nucleotide monomeric unit to
- 20 a second additional monomeric unit, at  
least one of the first and second addition-  
al monomeric units being a nucleotide  
monomeric unit.

2. A reagent as defined in claim 1, wherein said  
25 ligand is selected from a label, an intercalator, a metal  
chelator, drugs, hormones, proteins, peptide, haptens,

radical generators, nucleolytic agents, proteolytic agents, catalysts, specific binding substances, agents which modify DNA transport, and substances which alter nucleotide multimer solubility, and from a linking functional group which can be linked to any of the foregoing chemical moieties.

3. A non-nucleotide reagent suitable for preparing a nucleotide/non-nucleotide polymer, comprising:

- 10 (a) a non-nucleotide monomeric unit, having a ligand which is selected from a side arm with an attached chemical moiety, and from a protected linking arm which can be deprotected under non-adverse conditions so as to be capable of linking with a chemical moiety;
- 15 (b) a non-nucleotide, first coupling group and protected second coupling group, linked to the monomeric unit, the first coupling group which is capable of coupling the non-nucleotide monomeric unit to a first additional monomeric unit while the second one is substantially incapable of coupling, but can thereafter be deprotected under non-adverse conditions, so as to be capable of coupling the non-nucleotide monomeric unit to a second additional monomeric unit,
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- 25

at least one of the first and second monomeric units being a nucleotide monomeric unit.

4. A reagent as defined in claim 3, wherein said  
5 ligand is selected from a label, an intercalator, a metal chelator, drugs, hormones, proteins, peptides, haptens, radical generators, nucleolytic agents, proteolytic agents, catalysts, specific binding substances, agents which modify DNA transport, and substances which alter  
10 nucleotide multimer solubility, and from a linking functional group which can be linked to any of the foregoing chemical moieties.

5. A reagent as defined in claim 4 wherein the first and second coupling groups, are capable of coupling  
15 the non-nucleotide monomeric unit to a 5' hydroxyl and a 3' phosphate of a monomeric unit, respectively.

6. A reagent as defined in claim 5 wherein the non-nucleotide monomeric unit has an acyclic skeleton to respective ends of which the first and second coupling  
20 groups are linked.

7. A reagent as defined in claim 5 wherein the non-nucleotide monomeric unit has a skeleton to respective

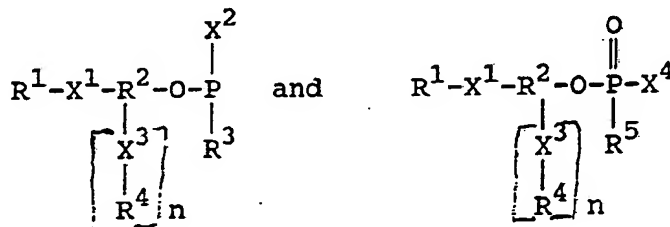
ends of which the first and second coupling groups are linked, the skeleton being a 1 to 20 atom chain.

8. A reagent as defined in claim 5 wherein the non-nucleotide monomeric unit has a skeleton to respective  
5 ends of which are linked the coupling groups, the skeleton being an acyclic hydrocarbon chain of from 1 to 10 carbon atoms.

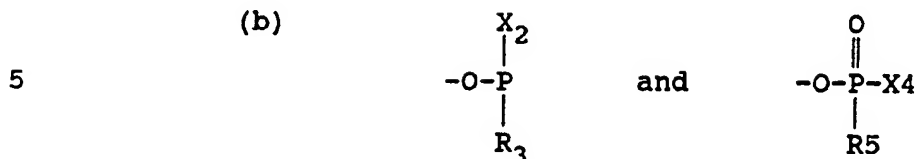
9. A reagent as defined in claim 5 wherein the non-nucleotide monomeric unit has a skeleton to respective  
10 ends of which are linked the coupling groups, and wherein the linking arm is a 1 to 25 atom chain extending from the skeleton.

10. (amended) A reagent as defined in claim 3  
selected from one of the compounds having the formula:  
15 wherein:

20



(a)  $R_2$  = a skeleton of the non-nucleotide monomeric unit;



are first coupling groups in which:

$X_2$  = halogen or substituted amino

$X_4$  = halogen; amino; or  $O^-$

$R_3$  = alkyl; alkoxy; or phenoxy

$R_5$  = alkyl; alkoxy; or aryloxy or may be  
H only if  $X_4 = O^-$

(c)  $R_1 - X_1 -$  is the protected second coupling  
group in which:

$X_1$  = O; S; NH; or HN=N-

$R_1$  = the protecting group cleavable under  
coupling group deprotecting condi-  
tions to recover the second coupling  
group  $H-X_1-$ ;

(d) n is an integer;

(e)  $X_3-R_4-$  is the ligand.

11. (amended) A reagent as defined in claim 10  
wherein:

$X_2$  = Cl; or secondary amino

$R_2$  = Chlorophenoxy; methoxy; ethoxy; or beta-  
cyanoethoxy



X4 = Cl; secondary amino; or O<sup>-</sup> when R<sub>5</sub> =  
H

R5 = methoxy; ethoxy; monochlorophenoxy; or  
beta-cyanoethoxy; or may be H only  
if X4 = O<sup>-</sup>

5  
12. A reagent as defined in claim 11 wherein the  
ligand is a protected linking arm in which X3 is an  
alkylamino linked to R2 by a C- and to R4 by N-, and in  
which R4 is selected from trifluoroacetyl and 9-fluorenyl-  
10 methoxycarbonyl.

13. A reagent as defined in claim 11 wherein R<sub>2</sub> has  
a secondary carbon linked to the -O-.

14. A reagent as defined in claim 10, wherein the  
secondary amino groups are selected from dialkylamino, and  
15 heterocyclic N-amines.

15. (amended) A reagent as defined in claims 10,  
11, or 14, wherein R<sub>1</sub> = triphenylmethyl or an alkoxy  
derivative thereof.

16. (amended) A reagent as defined in claim 10, 11  
20 or 14, wherein X<sub>1</sub> = O and R<sub>1</sub> = triphenylmethyl or an  
alkoxy derivative thereof.

17. A reagent as defined in claim 10, 11 or 14 wherein  $X_1 = O$  and  $R_1 = \text{dimethoxytriphenylmethyl}$ .

18. (amended) A method of preparing a substituted nucleotide from a reagent having:

- 5 (a) a non-nucleotide monomeric unit; and
- (b) a ligand linked to the non-nucleotide monomeric unit, and selected from a side arm with an attached chemical moiety, and from a linking arm to which a chemical moiety can be linked under non-adverse conditions; the method comprising coupling the non-nucleotide monomeric unit under non-adverse conditions, to a nucleotide monomeric unit and to one of a second monomeric unit and a solid support.
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19. (amended) A method of preparing a substituted nucleotide from a reagent having:

- (i) a non-nucleotide monomeric unit having a ligand, selected from a side arm with an attached chemical moiety and from a protected linking arm to which a chemical moiety can be linked; and
- 20 (ii) a first coupling group and a protected second coupling group linked to the non-nucleotide monomeric unit;
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the method comprising, under non-adverse conditions:

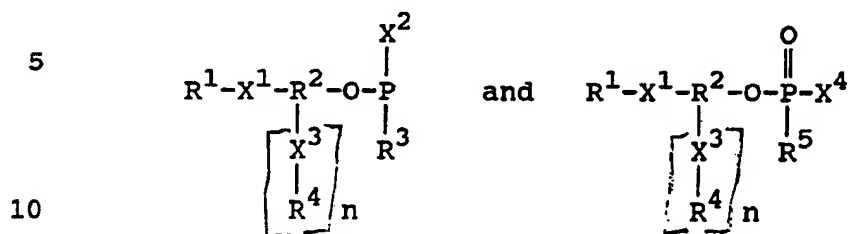
- 5 (a) first coupling the non-nucleotide monomeric unit to a first additional monomeric unit through the first coupling group;
- (b) then deprotecting the second coupling group;
- 10 (c) then coupling the non-nucleotide monomeric unit through the second coupling group, to a second additional monomeric unit;
- at least one of the first and second monomeric units being a nucleotide monomeric unit while the other one is selected from a monomeric unit and a solid support.

20. A method as defined in claim 18 or 19, wherein  
15 both the first and second additional monomeric units are nucleotide monomeric units.

21. A method as defined in claim 18 wherein the  
first and second coupling groups, are capable of coupling  
the non-nucleotide monomeric unit to a 5' hydroxyl and a  
20 3' phosphate of a nucleotide monomeric unit, respectively.

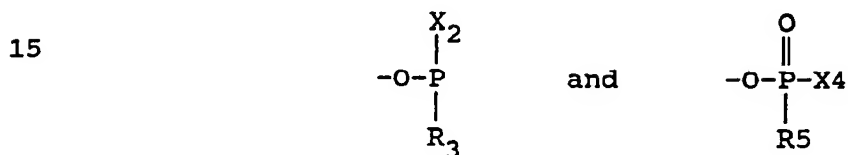
22. A method as defined in claim 19 wherein the non-nucleotide monomeric unit has an acyclic skeleton to  
respective ends of which the first and second coupling  
groups are linked.

23. (amended) A method as defined in claim 19 wherein the reagent is selected from one of the compounds having the formula:



wherein:

(a)  $R_2$  = a skeleton of the non-nucleotide monomeric unit;



are first coupling groups in which:

20  $X_2$  = halogen or substituted amino

$X_4$  = halogen; amino; or  $O^-$

$R_3$  = alkyl; alkoxy; or phenoxy

$R_5$  = alkyl; alkoxy; or phenoxy; or

may be H only if  $X_4 = O^-$

25 (c)  $R_1-X_1-$  is the protected second coupling group in which:

$X_1$  = O; S; NH; or HN=N-

$R_1$  = the protecting group cleavable  
under coupling group  
deprotecting conditions to  
recover the second coupling  
5 group H-  $X_1$ -;

(d) n is an integer;

(e)  $X_3$ - $R_4$ - is the ligand

24. A method as defined in claim 23, wherein said  
ligand is selected from a label, an intercalator, a metal  
10 chelator, and from a linking functional group which can be  
linked to one of a label, an intercalator, and a metal  
chelator.

25. (amended) A method as defined in claim 24  
wherein:

15  $X_2$  = Cl; or secondary amino

$R_3$  = chlorophenoxy; methoxy; ethoxy; or  
beta-cyanoethoxy

$X_4$  = Cl; secondary amino; or  $O^-$  when  $R_5$ =  
H

20  $R_5$  = methoxy; ethoxy; monochloophenoxy;  
beta-cyanoethoxy; or may be H only  
if  $X_4 = O^-$

26. A method as defined in claim 24 wherein the secondary amino groups of the reagent are selected from dialkylamino and heterocyclic N-amines.

27. A method as defined in claim 23 or 26 wherein  $R_1$  of the reagent is triphenylmethyl or an alkoxy derivative thereof.

28. A method as defined in claim 23 or 26 wherein the reagent  $R_1$  = dimethoxytriphenylmethyl.

29. A method as defined in claim 23 or 26 wherein the reagent has  $X_1 = O$  and  $R_1$  = dimethoxytriphenylmethyl.

30. A method as defined in any of claims 23 to 26 additionally comprising coupling further monomeric units to the coupled nucleotide/non-nucleotide polymer, to produce a polymer with a nucleotide sequence which is complementary to at least a portion of a target nucleotide sequence, but which complementary nucleotide sequence has at least one non-nucleotide monomeric unit disposed therein, then hybridizing the nucleotide/non-nucleotide polymer sequence with the target sequence.

31. (amended) A composition containing a single sequence nucleotide/non-nucleotide polymer as the majority

of all nucleotide/non-nucleotide polymers present, the single nucleotide/non-nucleotide polymer comprising:

- 5 (a) a non-nucleotide monomeric unit having linked thereto a ligand selected from a side arm with an attached chemical moiety, and from a linking arm which can be linked to a chemical moiety under non-adverse conditions;
- 10 (b) a first nucleotide monomeric unit coupled to the non-nucleotide monomeric unit; and
- (c) an additional monomeric unit coupled to the non-nucleotide monomeric unit.

32. A reagent as defined in claim 31, wherein said ligand is selected from a label, an intercalator, a metal chelator, and from a linking functional group which can be linked to one of a label, an intercalator and a metal chelator.

33. (amended) A composition as defined in claim 31 or 32 wherein the additional unit is a nucleotide monomeric unit.

34. (amended) A composition as defined in claim 31 or 32 wherein the additional monomeric unit is a non-nucleotide monomeric unit, which is coupled to another non-nucleotide monomeric unit.

35. (amended) A composition containing a nucleotide non-nucleotide polymer comprising:

- 5 (a) a non-nucleotide monomeric unit, having linked thereto a ligand selected from a side arm with an attached chemical moiety linked to the skeleton through a saturated linker group, and from a saturated linking arm which can be linked to a chemical moiety under non-adverse conditions;
- 10 (b) a first nucleotide monomeric unit coupled to the non-nucleotide monomeric unit; and
- (c) an additional monomeric unit coupled to the non-nucleotide monomeric unit.

36. A composition as defined in claim 35, wherein  
15 said ligand is selected from a label, an intercalator, a metal chelator, and from a saturated linking arm which can be linked to either of a label and an intercalator.

37. A composition as defined in claim 36 wherein the ligand group is the saturated linking arm.

20 38. A composition as defined in claim 34 wherein the non-nucleotide monomeric unit has a skeleton to the ends of which are linked the coupling groups, and wherein the ligand comprises a label linked to the skeleton through



the linking group which is selected from an alkylamino and alkylthio.

39. A composition as defined in claim 37 wherein the linking arm is selected from an alkylamino and an  
5 alkythio.

40. A composition as defined in claim 35, 37 or 39 wherein the non-nucleotide monomeric unit has a skeleton to the ends of which are linked the coupling groups, the skeleton being an acyclic hydrocarbon chain having from 1  
10 to 20 carbon atoms.

41. A composition as defined in claim 35 wherein the label is a chemiluminescent acridinium ester.

42. A composition as defined in claim 36 wherein the intercalator is an acridinium ester.

15 43. (amended) A composition as defined in claim 35 wherein the label is selected from biotin, fluorescein, dinitrobenzene, rhodamine, and Texas Red.

44. A composition as defined in claim 35 wherein the intercalator is selected from psoralen, acridine, acridi-  
20 nium salts, acriflavins, and ethidium.

45. A polymeric probe comprising a plurality of nucleotide monomeric units, and at least one acridinium ester moiety linked to a corresponding monomeric unit of the probe.

5        46. A probe as defined in claim 45 wherein the acridinium ester label is a chemiluminescent acridinium ester label.

47. A method of making a probe, comprising linking at least one acridinium ester moiety to a corresponding  
10 monomeric unit of a polymer having a plurality of nucleotide monomeric units.

48. A method as defined in claim 47 wherein the acridinium ester label is a chemiluminescent acridinium ester label.

15        49. (new) A reagent as defined in claims 10, 11, 24, 25, 31, or 35, wherein the ligand is a protected linking arm in which  $R_4$  is the linking arm and  $X_3$  is the protecting group.

50. (new) A reagent as defined in claims 10, 11,  
20 24, 25, 31, or 35, wherein the ligand is a protected linker arm in which  $X_3$  is an alkylamino linking arm linked

to  $R_2$  by a C- and to  $R_4$  by N-, and  $R_4$  is the protecting group.